171* Trypsin and neutrophil elastase regulate CFTR expression and function in cystic fibrosis

M. Le Gars[1,2], D. Descamps[1], N. Bakouh[3], G. Planelles[3], S. Hong[4], P. Boulanger[4], M. Huerre[1], M. Chignard[1], J.-M. Sallenave[1,2]. [1]*Institut Pasteur, Paris, France;* [2]*Université Paris VII, Paris, France;* [3]*INSERM U845, Université Paris-Descartes, Paris, France;* [4]*CNRS FRE-3011, Université de Lyon 1, Lyon, France*

Introduction: Cystic fibrosis (CF) is a genetic disease caused by mutations in the CF transmembrane conductance regulator (CFTR). Defective membrane mutant CFTR (ΔF508) expression is instrumental in mediating lung inflammation. Lung stromal and inflammatory cells secrete serine-proteases which have well described deleterious effects on the integrity of lung tissue. However, the effect of proteases on CFTR has not yet been addressed. The aim of the present study was to characterize the effect of tissue (trypsin-4) and neutrophil-derived (Elastase or NE) serine-proteases on CFTR both at structural and functional levels.
Results: We show here in bronchial and alveolar epithelial cells that NE and trypsin-4 up-regulate IL-8 production 1.3 to 1.5 fold in CF cells (over-expressing ΔF508 CFTR), respectively, when compared to WT cells. This induced IL-8 up-regulation was correlated with the NE (but not trypsin-4) generation of a cleaved 95 kDa CFTR molecular species, suggesting that the IL-8 pro-inflammatory phenotype is exacerbated by CFTR degradation.
In WT CFTR-transfected *Xenopus* oocytes, Forskolin+IBMX enhanced the slope of linear shaped I/V curves, consistent with the functional expression of CFTR. In WT CFTR-oocytes also incubated with NE, increase in conductance was 2 to 2.5 fold lower.
Conclusions: We demonstrate here that while both serine-proteases NE and trypsin-4 up-regulate IL-8 in bronchial and alveolar epithelial cells, only NE is able to significantly degrade the WT CFTR protein. This degradation also occurred with ΔF508 CFTR, suggesting that NE may exacerbate the CFTR phenotype in CF. We are currently dissecting the mechanisms involved.

172* A role for cathepsin S in the pathogenesis of cystic fibrosis lung disease

S. Weldon[1], J.S. Elborn[1], C.J. Scott[2], C.C. Taggart[1]. [1]*Queens University Belfast, Centre for Infection and Immunity, Belfast, United Kingdom;* [2]*Queen's University Belfast, School of Pharmacy, Belfast, United Kingdom*

The pathogenesis of lung disease in cystic fibrosis (CF) has not been fully elucidated; however, neutrophil-dominated inflammation is thought to play a major role. Nonetheless, a number of proteases produced by other cells in the lung may also play a pivotal role in CF lung damage. Human lysosomal cysteine proteases are a family of proteases that have been relatively unexplored in the area of CF lung disease. We have shown that cathepsin S activity is increased in CF BAL fluid, compared with that from healthy individuals. In addition to lung tissue degradation, cathepsins have been found to contribute significantly to the destruction of host defence proteins such as SLPI, β-defensins and lactoferrin. These findings indicate a role for cathepsin S in the diminution of the lung antiprotease and antimicrobial screen possibly leading to lung destruction and favouring conditions for bacterial infection. We have identified epithelial cells as a source of cathepsin S in the CF lung, with the demonstration that CF bronchial and tracheal epithelial cell lines (CFBE41o$^-$ and CFTE29o$^-$) secrete significantly more active cathepsin S than normal cells (16HBE14o$^-$ and 9HTEo$^-$) in the absence of proinflammatory stimulation. On the basis of our results to date, we postulate that upregulated cathepsin S plays an important role in CF lung disease and we are currently investigating reasons for this upregulation of cathepsin S in CF epithelial cells. This data will shed valuable light on the role of cathepsin S in CF, an area that has been overshadowed to date, and may open up new avenues for exploration in the search for an effective therapeutic target in CF lung disease.

173* Genetic and pharmacological evidence for critical role of macrophage elastase in emphysema formation in βENaC-overexpressing mice

S. Diemer[1,2], J. Trojanek[1,2], S. Schubert[1,2], A. Cobos[2,3], J. Schatterny[1], S. Hirtz[1], C. Schultz[2,3], M.A. Mall[1,2]. [1]*Division of Ped. Pulmonology and CF Center, Dept. of Pediatrics III, University of Heidelberg, Heidelberg, Germany;* [2]*EMBL-University of Heidelberg Molecular Medicine Partnership Unit, Heidelberg, Germany;* [3]*EMBL, Dept. of Cell Biology and Biophysics, Heidelberg, Germany*

Increased airway Na^+ absorption and airway surface liquid (ASL) depletion cause airway mucus obstruction, chronic airway inflammation and emphysema, which are the main characteristics of cystic fibrosis (CF)-like lung disease in βENaC-overexpressing (βENaC-Tg) mice. The aim of this study was to investigate the mechanism underlying emphysema formation in this murine model of CF lung disease. Screening of mRNA levels of proteases/antiproteases in the lungs of βENaC-Tg mice compared to wild-type littermates showed a marked increase of macrophage elastase (also known as MMP12) transcript expression. By performing longitudinal measurements of lung volumes and mean linear intercepts, we demonstrated that the onset of emphysema formation occurred in the first days of life and that distal airspace enlargement progressed in older βENaC-Tg mice. To elucidate the role of MMP12 in emphysema formation, we crossed βENaC-Tg with MMP12 deficient mice and found that double-mutant βENaC-Tg/MMP12$^{-/-}$ were protected from emphysema formation. Further, daily treatment with the MMP inhibitor GM 6001 prevented emphysema formation in βENaC-Tg mice. Our results indicate that ASL depletion leads to macrophage activation and elevated secretion of MMP12 and identify MMP12 as cause of emphysema in βENaC-Tg mice. These results may offer novel therapeutic opportunities for the treatment of CF lung disease.
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174* Flagellin/TLR5-dependent modulation of alveolar macrophage and epithelial cell activity by the antimicrobial molecule trappin-2

D. Descamps[1], M. Le Gars[1], V. Balloy[1], R. Ramphal[2], M. Chignard[1], J.-M. Sallenave[1,3]. [1]*Institut Pasteur, INSERM U874, Defence Innée et Inflammation, Paris, France;* [2]*University of Florida, Gainesville, FL, United States;* [3]*Université Paris VII, Paris, France*

Epithelial-derived endogenous antimicrobial molecules such as defensins or trappin-2 (T2) have the potential to affect the phenotype of phagocytic myeloid cells (macrophages, neutrophils, dendritic cells), by modifying their differentiation, chemotactic properties and activation in different lung pathologies. Recently, it has been shown that T2 can improve the clearance of *Pseudomonas aeruginosa* (*P.a*) by alveolar macrophages (AM) (Wilkinson *et al*. Am. J. Pathol, 2009, 174:1338–46). The aim of the present study is to study T2 opsonizing effect on the clearance of *P.a* by AM and a potential modulation on cytokine productions by AM and lung epithelial cells stimulated with *P.a.*
We have measured the early clearance of PAK (a wild-type *P.a.* strain), PAKD*fliC* (a flagellum-deficient PAK) or PAKL88 (a PAK strain with a TLR5-binding site-deficient flagellum), pre-incubated or not with T2 (100 nM, a sub-lytic concentration), by murine AM (MH-S cell line). We confirm that T2 enhances the early clearance of PAK by AM but not that of PAKD*fliC* or PAKL88. In addition, T2-pre-incubated PAK but not PAKD*fliC* increases AM or lung epithelial cells TNF-a and IL-8 secretions. Moreover, epithelial cells stimulated with PAK-flagellin pre-incubated with T2 secreted higher levels of IL-8, when compared with cells incubated with flagellin alone.
Our data suggest that T2, by 'opsonising' *P.a* and by increasing cytokine production of AM or lung epithelial cells may constitute an important modulator of innate immune responses. Moreover, T2 activity requires *P.a* flagellin-TLR5 interaction. We are currently assessing whether T2 could improve the defective bacterial clearance observed in cystic fibrosis.